counteracting the formation of disulfide bridges to maintain proteins in a reduced state.

It therefore was completely unexpected when recently a machinery was identified in the intermembrane space of mitochondria that facilitates the import of proteins in a reaction that relies on the oxidative folding of proteins [1–3]. This machinery consists of two components which are highly conserved and essential for viability: the oxidoreductase Mia40 and the sulfhydryl oxidase Erv1. In vitro and in vivo data suggest that Mia40 directly interacts with incoming substrate proteins, thereby introducing structural disulfide bonds [4–6]. Mia40 specifically interacts with hydrophobic regions of intermembrane space proteins and supports their import and folding in a chaperone-like manner.

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4P1

Respectively, Asp, PPi and Na^+-pump emerged before Glu, ATP and H^+-pump

H. Baltscheffsky, N. Holm
Department of Biochemistry and Biophysics, Stockholm University, Sweden
Department of Geological Sciences, Stockholm University, Sweden
E-mail: herrick@dbb.su.se

Asp, in contrast to Glu, appears to have been among the four first proteinaceous amino acids to participate in the coded peptide synthesis in connection with early life on the Earth (Eigen, Trifonov). The recently presented 3-D picture of a membrane-bound H^+-pumping inorganic pyrophosphatase [1] strongly supports earlier suggestions [2] about a remaining primitivity of amino acid sequences, involving many Asps (plus Mg^{2+} [3]) for the binding of PPi to the enzyme. We have recently claimed that PPi appeared before ATP and Na^+ before H^+ [4] based on earlier data [5,6]. The 3-D picture given indicated a H^+-pumping mechanism completed through a series of conformational changes driven primarily by PPi hydrolysis energy. This interpretation of the 3-D picture invites a comparison with that of the ATP synthase, which with its several subunits and complex rotatory mechanism appears to further support the assumption that the less complex PPi system structure and reaction mechanism appeared earlier than that involving ATP. The still unknown details of an evolution from an early PPi system for bioenergy transformation to an ATP system (adding the possibility for adenylation) may involve a significant paradigm shift, an 'anastrophic shift' [7,8].

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4P2

Substrate binding in the mitochondrial ADP/ATP carrier

Elizabeth Cerson, Edmund Kunji
Medical Research Council, Mitochondrial Biology Unit, Wellcome Trust/MRC building, Hills Road, Cambridge CB2 0XY, UK
E-mail: ec430@mrc-mbu.cam.ac.uk

The ADP/ATP carrier in the inner mitochondrial membrane exchanges cytosolic ADP and matrix ATP. Whilst the exact location of the substrate binding site has been proposed by symmetry analysis, distance constraints [1] and molecular dynamics simulations [2], it has yet to be structurally and biochemically defined. Computer modeling of ADP binding to a fungal ADP/ATP carrier was performed to determine the residues that were potentially interacting with the substrate, showing an arrangement that agreed with published data. Mutations of these residues were engineered, and the mutant proteins were expressed in *Lactococcus lactis* and tested in transport assays to determine how these mutations affect the transport function. In combination with substrate binding assays, this information could lead to the production of a mutant that can bind ADP, but not transport it, possibly allowing for the structure of the substrate-bound ADP/ATP carrier to be solved.

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